3940-Pos Board B668
Strain-Based Mechanism of Kinesin ATPase
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Kinesin family motors have a highly conserved set of residues at the ATP binding pocket, yet members differ in their ATPase rate. The ATPase rate should thus be determined not only by conserved residues in contact with ATP, but allosterically by other parts of the motor head. We perform all-atom molecular dynamics (MD) simulations of several different kinesin structures in ATP-like (ATP analog) states with and without binding to tubulin. Model of the kinesin-tubulin complex was built from either recent x-ray structure (PDB 4HNA) or sub-nanometer resolution cryo-EM structures. We find that the motor head has an inherent tendency to carry out a 'see-saw' motion so as to rotate into the ADP-like state. This tendency results in generation of a pulling force on the gamma phosphate of ATP by the switch-I domain. Differences in ATPase rates among kinesin families are thus likely to be caused by different amount of torque generated by the tendency of the motor head to rotate. In this process, the microtubule appears to increase the torque by holding the kinesin motor head in a strained conformation.

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Development of Molecular Shuttle Regulated by External Stimulation Utilizing Kinesin ATP Driven Motor
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Kinesin is known as a dimeric motor protein, which carries cellular cargoes along microtubules by hydrolyzing ATP. Its structure and the molecular mechanism of energy transduction to move along microtubule are well studied. The kinesin has many possibility of application to the molecular machines. Previously, we introduced photochromic molecules into the functional key region of the kinesin as a photoswitch and tried to control the function of kinesin by ultraviolet (UV) and visible (VIS) light irradiations. The kinesin mutant S275C modified with thiol reactive azobenzene derivative exhibited photocontrolled ATPase activity correlating to photo isomerization. We have also demonstrated that kinesin fused with CaM at the C-terminal binds reversibly to M13-Qdots in a calcium dependent manner. As mentioned above, kinesin works as a nanodevice to be a component of bionanomachines. In this study, we tried to prepare the molecular shuttles regulated by external stimulation utilizing kinesin and other functional proteins. We designed the two chimeric kinesins. One is the kinesin motor domain (K355) fused with calmodulin (CaM) and the other is K355 fused with calmodulin target peptide M13. The chimeras were expressed by E. coli. and purified by Co-Chelate column. These kinesin chimeras showed normal ATPase activities. K355-CaM bound to K355-M13 in a calcium dependent manner and formed dimer. The dimer composed of the two chimeras induced exhibited motor activity to induce microtubule gliding. Moreover, we also tried to prepare the chimeric kinesins, K355-CaM-split GFP C-terminal domain and K355-split GFP N-terminal domain to be dimerized and fluorescently labelled spontaneously.

3942-Pos Board B670
Collective Motions and Dynamical Couplings in the Kinesin Motor Domain
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Kinesin motor proteins transport cargo along microtubule tracks to support essential cellular functions including cell growth, axonal signaling and the separation of chromosomes during cell division. All kinesins contain one or more conserved motor domains that modulate binding and movement along microtubules via cycles of ATP hydrolysis. Important conformational transitions occurring during this cycle have been characterized with extensive crystallographic studies. However, the link between the observed conformations and the mechanisms involved in conformational change and microtubule interaction modulation remain unclear. Here we describe a comprehensive principal component analysis of 114 available motor domain crystallographic structures supplemented with extensive unbiased conventional and accelerated molecular dynamics simulations. This combined approach quantitatively assess the structural and dynamical features of distinct motor domain conformations, characterizes the response to nucleotide hydrolysis and microtubule binding, and probes the apparent allosteric link between functional sites. Simulations of unprecedented length for this system reveal the atomic details of large scale conformational transitions (most notably of the microtubule binding α4-α5, loop8 subdomain, α2b-β4-β6-β7 motor domain tip and loop5 regions), as well as novel dynamical couplings, of distal nucleotide and microtubule binding sites (mediated by loop7 and loop13). Our results also indicate that the crystallographically reported ATP and ADP-like conformations of kinesin in isolation are intrinsically accessible regardless of nucleotide state. Comparison with kinesin-tubulin simulations support a model where complex formation and neck-linker docking leads to a tighter coupling of the microtubule and nucleotide binding regions. Furthermore, mutational simulations highlight sites potentially critical for conformational transitions and allosteric coupling that are prime targets for experimental study.

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Introducing a Kinesin-Inspired Nanomotor Concept
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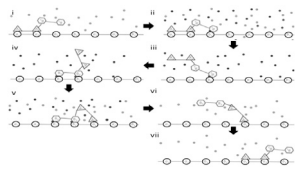
Based on a recent approach to understanding protein-based molecular motors (1), we present computer simulations of a novel nanomotor concept dubbed the Synthetic Kinesin Analog Motor Protein (SKAMP). SKAMP is a purely diffusive linear motor consisting of four ligand gated DNA-binding (repressor) proteins of two types, A and B, linked by three rigid coiled-coils ('rods') to form a complex A1-A2-B2-B1, the length of the central rod being shorter than the outer two. Directional stepping along a periodic DNA track is maintained by a temporally periodic external chemical supply. Due to the shorter central rod, SKAMP makes use of a mechanism analogous to that used by kinesin which involves docking of a neck linker onto a motor domain. This mechanism allows kinesin to reduce its diffusional search time for a binding site. We use coarse-grained Langevin Dynamics simulations in the overdamped limit to study the detailed motion of SKAMP when subject to a load force, SKAMP acting as a shuttle and the increase of SKAMP's performance by external feedback.



1. Bromley et al. HFSP Journal 3 204-213 (2009).

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Simulations of Neck-Linker Modified and One Head Loaded Kinesins
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Recently, Czovek et al. established a complete, thermodynamically consistent kinetic model for the two-headed homodimeric motor protein, kinesin. Computational simulations based on the model justified the crucial role of the conformational changes of the neck-linkers (NLs, the peptide chains connecting the two motor domains to the stalk) in the directional movement and force-generation of conventional kinesin. The model was able to reproduce a large number of experimental data (speed, dwell time distribution, randomness, processivity, hydrolysis rate, etc.) astonishingly well under normal as well as under highly unphysiological conditions. Moreover, it enabled a more detailed deconvolution of the mechanochemical cycle than it is experimentally possible. Having such a powerful model, we have applied it to modified versions of the wild-type kinesin, and reproduced (i) the speeds, processivities, and ATP consumption rates of NL modified kinesin; and (ii) the force-velocity relationship of the one-head-pulled kinesin. The good agreement between the simulations and the experiments further justify the legitimacy of the model, which thus provides a detailed understanding of the experimental observations and the basic mechanism of the operation of kinesin.

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Strain-Dependent Regulation of the Kinesin-1's Catalytic Activity as Studied by Disulfide-Crosslinking of the Neck Linker
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Kinesin-1 walks along microtubules by alternately hydrolyzing ATP and moving two motor domains. Several recent studies have suggested that the strain developed through the neck linker is essential for the coordination between two motor domains, although the mechanism for the regulation of the motor domain's activity remains unknown. At the last annual meeting we employed disulfide-crosslinking between cysteine residues introduced into the neck linker and the motor domain to constrain the neck linker of a monomer in the forward or backward extended conformation, and using single molecule fluorescent observation we showed that detachment rate from the microtubule dramatically decreased